ARGINASE STUDIES ON NORMAL AND TUMOR-BEARING ANIMALS

BY

GEORGE HILGARD BURNETT

A.B., University of Illinois, 1947

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMISTRY IN THE GRADUATE COLLEGE OF
THE UNIVERSITY OF ILLINOIS, 1950

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INTRODUCTION

Kossel and Dakin discovered arginase in 1901 (1). Investigation has revealed it to be a key enzyme in mammalian urea formation (2, 3). It exhibits first order kinetics when activated by certain heavy metals and is extremely stable in storage.

Arginase was determined by the Greenstein method (4) in the present work. Samples of buffered enzyme solution (activated by manganese chloride) were incubated with arginine monohydrochloride for selected periods of time. Then the reaction was stopped by heat and acetic acid. The urea formed by the arginine hydrolysis was converted to ammonium salts by urease. Then the sample was placed in a bubbler system and its ammonia was aerated into an acid tube and determined (by the Nessler procedure). From the experimental data, the first order reaction constant of the enzyme (at this dilution) was calculated. For comparison purposes, this reaction constant was divided by the milligrams of nitrogen in the enzyme sample (determined by a micro-Kjehldahl procedure.) The final unit was $Kx10^2/Mg.\ N.$ (Normal values averaged 37 ± 5).

In an effort to make this unit more "physiological" an arginase coefficient was calculated. It was the product of the K value and liver weight in grams divided by the body weight in grams (minus the tumor weight, if present.)

The purpose of this work was to determine how the liver arginase of mice and rats responded to different conditions of stress, including tumor growth. It has been reported by Greenstein et al. (4) that there is often a marked lowering of liver arginase activity associated with the development of certain hepatic (and extrahepatic) tumors in the rat. It seems desirable to emphasize the importance of a careful selection of activity units in experiments of this type. The data reported here have been subjected to statistical analysis.

MOUSE STUDIES

C58 leucemic mice (5) showed normal liver arginase K levels in spite of lymphocytic infiltration of various organs including the liver. Certain C58 animals with fatty livers exhibited low liver arginase K levels, but animals of both types had normal arginase coefficients.

Hall tumor mice (6) carrying a rapidly growing (14 to 20 days) undifferentiated sarcoma showed essentially normal liver arginase K levels. The arginase coefficients of a first series were normal, but those of a second series were somewhat higher than normal.

Irradiated mice had normal liver arginase K levels, but showed increased arginase coefficients. On the other hand, starved mice exhibited high arginase K levels, but normal arginase coefficients.

The over all picture seems to indicate that the mouse retains and sometimes increases his liver arginase when he is put under stress.

RAT STUDIES

Normal rat livers showed low arginase activity in the first three weeks of life.

In the course of these studies a spontaneous rat tumor, which was thought to be of mammary origin, was transplanted subcutaneously into the *latissimus dorsi* region of young rats. The tumor developed rapidly on transplant and has been carried through 11 rat transfer generations. At present the tumor grows to a considerable terminal size (50 g. in a 150g. rat) and kills in about 40-50 days. Recent histological examination suggests that the tumor is a mixed tumor with certain characteristics of an adenocarcinoma.

After transplant with this adenocarcinoma, rats gained weight rapidly, stabilized, and then showed sharp weight decline. Death usually followed. Food intake closely paralled the rise and fall of animal weight. Tumor growth continued at a uniform rate through the whole period. These animals showed normal or slightly decreased liver arginase K levels. Their arginase coefficients were all normal. There was no correlation between blood arginase as determined microbiologically and liver arginase levels.

Starved rats showed normal liver arginase. (Neither the liver arginase K levels nor the arginase coefficients were statistically significant from the control animals.)

The results indicate that rat liver arginase levels are very stable when calculated as above.

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VITA

The author was born on September 28, 1925 in Pittsburgh, Pennsylvania, where he attended elementary school. In 1939 his family moved to Rome, Georgia, where he attended Rome High School. From June, 1942, to June, 1944, he attended Oberlin College and then entered service. Returning from the Army (June, 1946) he entered the University of Illinois in September, 1946, and received his A.B. degree in August, 1947. He entered the Graduate College of the University of Illinois in September, 1947, and held a half-time teaching assistantship for the years, 1949 and 1950.

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